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Review

C_4-dicarboxylate carriers and sensors in bacteria

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Abstract

Bacteria contain secondary carriers for the uptake, exchange or efflux of C_4-dicarboxylates. In aerobic bacteria, dicarboxylate transport (Dct)A carriers catalyze uptake of C_4-dicarboxylates in a H^+- or Na^+-C_4-dicarboxylate symport. Carriers of the dicarboxylate uptake (Dcu)AB family are used for electroneutral fumarate:succinate antiport which is required in anaerobic fumarate respiration. The DcuC carriers apparently function in succinate efflux during fermentation. The tripartite ATP-independent periplasmic (TRAP) transporter carriers are secondary uptake carriers requiring a periplasmic solute binding protein. For heterologous exchange of C_4-dicarboxylates with other carboxylic acids (such as citrate:succinate by CitT) further types of carriers are used. The different families of C_4-dicarboxylate carriers, the biochemistry of the transport reactions, and their metabolic functions are described. Many bacteria contain membraneous C_4-dicarboxylate sensors which control the synthesis of enzymes for C_4-dicarboxylate metabolism. The C_4-dicarboxylate sensors DcuS, DctB, and DctS are histidine protein kinases and belong to different families of two-component systems. They contain periplasmic domains presumably involved in C_4-dicarboxylate sensing. In DcuS the periplasmic domain seems to be essential for direct interaction with the C_4-dicarboxylates. In signal perception by DctB, interaction of the C_4-dicarboxylates with DctB and the DctA carrier plays an important role.

Keywords: Fumarate/succinate transport; Dicarboxylate uptake carrier; Dicarboxylate transport A carrier; Antiport; Fumarate (succinate) sensor; Dicarboxylate uptake S; Dicarboxylate transport B; Two-component system; Histidine protein kinase

1. Introduction

C_4-dicarboxylates like succinate, fumarate, and malate and the C_4-dicarboxylic amino acid aspartate are metabolized by bacteria under aerobic or anaerobic conditions. In aerobic growth, the substrates serve as the carbon and energy source and become oxidized to CO_2 in the citric acid cycle. This metabolism requires *uptake* systems for the C_4-dicarboxylates. In anaerobic energy metabolism of most bacteria only C_4-dicarboxylates other than succinate are metabolized due to the lack of a functional citric acid cycle [1–3]. Fumarate, or malate and aspartate which can be easily converted to fumarate by dehydration or deamination, are used as electron acceptor in fumarate respiration. Succinate produced in fumarate respiration is excreted by *antiport* with fumarate (or malate, aspartate). *Shewanella* and some other bacteria catalyze fumarate reduction by a periplasmic enzyme without the need for fumarate uptake [4].

Abbreviations: Dcu, dicarboxylate uptake; Dct, dicarboxylate transport; $\Delta\Psi$, electrical potential across a membrane; TRAP, tripartite ATP-independent periplasmic transporter

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In many fermentation reactions succinate is formed from sugars. The succinate cannot be further metabolized and is excreted [5,6]. Thus enteric bacteria form up to 0.2 mol succinate per mol of glucose in the mixed acid fermentation. In citrate fermentation by *Escherichia coli* succinate is produced as an end product, and the bacteria contain a citrate:succinate antiporter [7,8].

A large number of anaerobic bacteria grow by decarboxylation of dicarboxylic acids as the energy source (reviewed in [9]). Thus *Propionigenium modestum* grows at the expense of succinate decarboxylation yielding propionate, and requires a succinate:propionate antiport. In malolactic fermentation, lactic acid bacteria decarboxylate malate and excrete lactate in a malate:lactate antiport [9,10]. In mitochondria and chloroplasts from eukaryotes various dicarboxylate carriers are found which link metabolic pathways in the mitochondria (citric acid cycle) and the cytoplasm (glycolysis, gluconeogenesis, glyoxylate cycle) and serve as carbon and [H]-shuttles between the compartments [11], including a succinate:fumarate antiport required in yeast for growth on ethanol or acetate [12].

This review will concentrate on the function of bacterial secondary carriers specific for C_4-dicarboxylates. Only CitT from *E. coli* catalyzing citrate:succinate antiport in citrate fermentation [8] will be discussed in context with the carriers specific for C_4-dicarboxylates.

The bacteria induce C_4-dicarboxylate metabolism only in the presence of external C_4-dicarboxylates [13–17]. Thus rhizobial strains use C_4-dicarboxylates supplied by the host as the major carbon and energy source during symbiotic growth. The corresponding pathways of C_4-dicarboxylate metabolism are induced by the substrates. In facultatively anaerobic bacteria capable of fumarate respiration, the enzymes of fumarate respiration are induced only in the presence of fumarate or other C_4-dicarboxylates. Thus the bacteria contain regulatory systems for sensing C_4-dicarboxylates available in the medium. After signal perception by the sensors and transmission to the cytoplasm, synthesis of the C_4-dicarboxylate metabolizing enzymes is controlled in response to the signal. Some of the sensors require C_4-dicarboxylate carriers for signal perception thus linking transport to the function of the chemosensors. The function of the sensory and regulatory systems responding to external C_4-dicarboxylates will be discussed.

2. Transport of C_4-dicarboxylates by bacteria

The C_4-dicarboxylate carriers have to fulfil a large number of different functions in bacteria, and catalyze symport (uptake or efflux) of the C_4-dicarboxylates with H^+ or Na^+, or homologous or heterologous antiport. The carriers can be grouped in different families which will be introduced first. Subsequently the characteristics of the carrier families will be shown. Many bacteria contain more than one C_4-dicarboxylate carrier. The need for different C_4-dicarboxylate carriers within one bacterium will be discussed in the last part of this section.

2.1. Families of C_4-dicarboxylate carriers

The C_4-dicarboxylate carriers from bacteria can be classified by protein sequence similarities in five coherent groups, the dicarboxylate transport (Dct)A, dicarboxylate uptake (Dcu)AB, DcuC, CitT, and the tripartite ATP-independent periplasmic transporter (TRAP) families (Fig. 1). Most of the carriers accept C_4-dicarboxylates (succinate, fumarate, malate) and C_4-dicarboxylic amino acids (aspartate) as substrates, but the K_D values for the various substrates may differ.

The DctA family comprises a large number of carriers from aerobic or facultatively anaerobic bacteria and catalyze uptake of C_4-dicarboxylates during aerobic growth. The DctA family is a subgroup of the 'dicarboxylate/amino acid:cation symporter' or DAACS family of carriers [18–20]. Members of the DAACS family are found in bacteria, archaea and eukaryotes and catalyze H^+ or Na^+ symport with C_4-dicarboxylates, dicarboxylic amino acids (aspartate, glutamate) or other amino acids. The DctA carriers from Gram-negative bacteria [21–24] and from the Gram-positive bacteria with high GC content (*Streptomyces coelicolor*, *Corynebacterium glutamicum* and *Mycobacterium tuberculosis*) form distinct subgroups of the DctA family. The DctA like protein YdbH from *Bacillus subtilis* [25], a Gram-positive bacterium with low GC content, on the other hand, is more similar to DctA from the

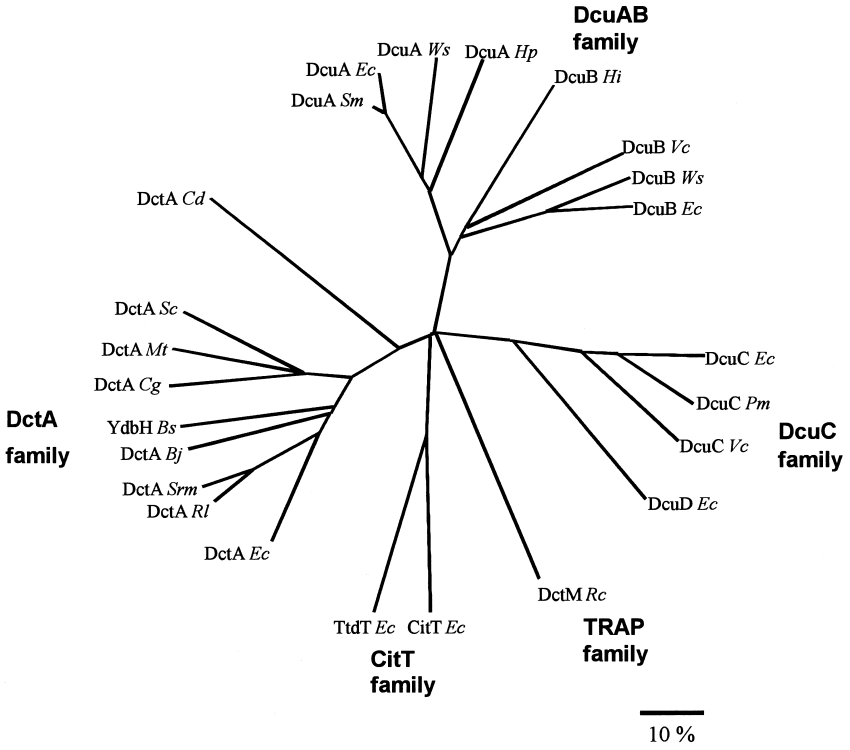

Fig. 1. Families of bacterial C_4-dicarboxylate carriers (DctA, DcuAB, DcuC, CitT, and TRAP), families based on comparison of the protein sequences. The distances give the differences in identical amino acid residues of the carriers or the corresponding (putative) gene products. For the comparison the Clustal W program was used (opening, end, extending and separation gap penalties: 10, 10, 0.05, and 0.05, respectively). Abbreviations of strains: *Bj*, *Bradyrhizobium japonicum*; *Bs*, *Bacillus subtilis*; *Cd*, *Corynebacterium diphtheriae*; *Cg*, *C. glutamicum*; *Ec*, *Escherichia coli*; *Hi*, *Haemophilus influenzae*; *Hp*, *Helicobacter pylori*; *Mt*, *Mycobacterium tuberculosis*, *Pm*, *Pasteurella multocida*; *Rc*, *Rhodobacter capsulatus*; *Rl*, *Rhizobium leguminosarum*; *Sc*, *Streptomyces coelicolor*; *Sm*, *Serratia marcescens*; *Srm*, *Sinorhizobium meliloti*; *Vc*, *Vibrio cholerae*; *Ws*, *Wolinella succinogenes*.

Gram-negative bacteria. DctA from *Corynebacterium diphtheriae* is only distantly related to the other DctA proteins.

The DcuAB carriers form an independent family present only in anaerobic and facultatively anaerobic bacteria capable of fumarate respiration [26–29]. The DcuA and DcuB carriers are capable of C_4-dicarboxylate exchange, uptake and presumably efflux, but operate preferentially as exchange or uptake carriers. The DcuAB (or Dcu [20]) family can be subdivided into the DcuA and DcuB subgroups.

The DcuC family is functionally similar to the DcuAB carriers and catalyzes exchange, uptake and efflux of C_4-dicarboxylates under anaerobic growth conditions [27], but the major function seems to be efflux. The DcuC family is well separated from the DcuAB family and contains only a small number of members which are all from enteric bacteria. DcuD [28] and gene products termed 'DcuE' in Table 2 represent separate subgroups of DcuC with unknown function.

The 'TRAP family' represents a novel family of secondary carriers composed of two membrane integral proteins and a periplasmic solute binding protein [30]. The C_4-dicarboxylate carrier DctPQM from *Rhodobacter capsulatus* has been characterized at the functional and genetic level [31,32]. Homologs of DctPQM are found in Gram-negative bacteria and archaea (*Achaeoglobus fulgidus*). The TRAP carriers are well separated from the other secondary carriers by their primary structure, subunit composition and mode of function.

The carboxylate:C_4-dicarboxylate antiporter family with the citrate:succinate antiporter CitT of *E. coli* [30] is a subgroup of the 'divalent anion:Na^+ symporters' (DASS) [19,20]. Carriers of this family are found in eukaryotes and bacteria, and mostly catalyze an exchange of di- and tricarboxylic acids.

Table 1
Molecular properties of the C_4-dicarboxylate carriers of *E. coli*

Carrier	Map position (min)	M_r (kDa)	Family	Transmembrane helices		Function
				Predicted	Measured	
DctA	79.3	45.3	DctA	10 or 12		uptake (aerobic)
DcuA	94.1	45.6	DcuAB	12 (14)	10	support for DcuB/C and for switch from DctA to DcuB/C
DcuB	93.7	47.8	DcuAB	12 (14)	10	exchange, uptake (anaerobic)
DcuC	14.1	48.3	DcuC	12		efflux, exchange, uptake (anaerobic)
DcuD (YhcL)	72.7	48.7	DcuC	12		silent?
CitT	13.9	52.9	CitT	12		citrate/succinate exchange (anaerobic)

The map position gives the location of the structural genes in the *E. coli* map. The function gives the transport modes for the different carriers (for details see text). The most important function is underlined.

Most of the DASS carriers are found in mitochondria or chloroplasts [11]. In bacteria only few members are known, such as the CitT carrier from *E. coli* catalyzing citrate:succinate antiport, and the putative tartrate:succinate antiporter of *E. coli* (*ttdT* gene) [33]. In contrast to DctA, the Dcu and DctPQM carriers, is CitT not restricted to C_4-dicarboxylates. CitT will be discussed briefly due to its role in C_4-dicarboxylate metabolism.

In addition to these C_4-dicarboxylate carriers a considerable number of other carriers are able to transport C_4-dicarboxylates in a side-reaction with low affinity. In mutant strains or under specific conditions these carriers can take over the function of the C_4-dicarboxylate carriers. Among those are the ketoglutarate:H^+ symporter (KgtP from *E. coli*) [34], a putative monocarboxylate carrier from *E. coli* [35], carriers from the 'tellurite:dicarboxylate transporter' (TDT) family, and from the 'citrate:cation symporter' (CCS) family [19,20].

2.2. The DctA carriers

DctA carriers or *dctA* like genes are found in aerobic bacteria which grow on succinate or other C_4-dicarboxylates. Facultative anaerobes like *E. coli* synthesize the DctA carriers only during aerobic growth [21]. In symbiotic *Rhizobium* strains which receive malate or other C_4-dicarboxylates from their plant host as the carbon source, DctA carriers are of particular significance [16,36–38]. Rhizobial strains deficient of *dctA* are defective in nitrogen fixation. The DctA carriers from Gram-positive bacteria like *Bacillus*, *Corynebacterium* or *Streptomyces* have not been characterized in much detail. The DctA carriers are cation (H^+ or Na^+):C_4-dicarboxylate symporters and are composed of about 450 amino acids and 10 or 12 putative transmembrane helices (Table 1).

Based on general signature sequences for the N-terminal and C-terminal parts of the Na^+:dicarboxylate family of carriers (PS00713 and PS00714 in Prosite database, http://www.expasy.ch), for the 18 H^+-dependent DctA carriers modified N- and C-terminal consensus sequences can be suggested:

(35) KPxGDxFxxLxKMxIxPxIF (54)

(291) VGLVxPTGYSFNLDGTxIYxFxAxxFxAQ (318)

The numbering refers to *E. coli* DctA, and the amino acid residues given in the one-letter code are present in $>94\%$ of the DctA sequences. The N-terminal consensus is part of the putative transmembrane helix 1 (aa 21–39) and the first periplasmic loop (aa 40–58) of the 12 transmembrane helix topological model of *E. coli* DctA. The C-terminal consensus comprises parts of a potential cytoplasmic loop (aa 291–310) and the subsequent transmembrane helix 9 (aa 311–329). Therefore the major parts of the consensus sequences are part of hydrophilic loops and contain substantial amounts of charged or polar amino acid residues. The consensus sequences for the putative Na^+-dependent DctA carriers are similar. However, in the N-terminal consensus of the latter, some residues are not (D_{39}) or less well (P_{36}) conserved. In the C-terminal consensus, L_{293}, V_{294} and Q_{318} are not or less well conserved.

2.2.1. Transport and energetics of DctA from Gram-negative bacteria

In *E. coli* DctA catalyzes the uptake of C_4-dicarboxylates (succinate, fumarate, malate), aspartate [39], and of the aromatic monocarboxylic acid orotate as a source for pyrimidine [40] in aerobic growth. *dctA* mutants show only poor growth on C_4-dicarboxylates [21].

Most kinetic studies on aerobic C_4-dicarboxylate uptake were performed with wild-type *E. coli*, but the data should be representative for DctA, since DctA is the major carrier in aerobic growth. The K_m for the C_4-dicarboxylates is about 10–30 μM, and V_{max} about 25–50 μmol/min/g dry weight [39]. The transport requires the electrochemical H^+ potential [41], and approximately two H^+ enter the cell with each C_4-dicarboxylate:

$$\text{succinate}^{2-}_{out} + 2H^+_{out} \rightarrow \text{succinate}^{2-}_{in} + 2H^+_{in} \quad (1)$$

In leguminous plants *Sinorhizobium*, *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium* are responsible for symbiotic nitrogen fixation. The host plant supplies succinate, malate and fumarate as the carbon and energy source to the rhizobial bacteroides in the root nodules. Rhizobial *dctA* mutants produce nodules which are symbiotically inactive in N_2 fixation and unable to transport C_4-dicarboxylates [38,42]. The kinetic properties of the DctA carriers are similar to those from *E. coli*. The K_m for the C_4-dicarboxylates is 2–15 μM, whereas that for aspartate is up to 10 mM, and the V_{max} is in the range of 1.5–100 μmol/min/g protein [43–45]. In the rhizobial uptake of orotate ($K_m = 1.7$ mM) also requires the DctA carrier [46].

2.2.2. DctA-dependent transport in Gram-positive bacteria

In *B. subtilis*, the *ydbH* gene product shows similarity to the DctA carriers of Gram-negative bacteria (Fig. 1), and inactivation of the gene causes loss of fumarate and succinate transport [25]. Malate presumably is transported by the *yufR* gene product, which shows similarity to malate permeases. The K_m values for the C_4-dicarboxylates were 4.3–13.5 μM in vesicles [47] and about 10-fold higher in intact cells [48]. The C_4-dicarboxylates are taken up by a Δp-dependent transport which is sensitive to uncouplers [49].

C. glutamicum contains a succinate uptake carrier which is presumably encoded by the *dctA* like gene (*orf2848*). The uptake has a high K_m for succinate (150 μM) and relatively low transport activities (up to 7 μmol succinate/min/g dry weight) [50]. Succinate uptake depends on the presence of Na^+ ions in the medium and on electrical potential across a membrane ($\Delta\Psi$). Thus DctA from *C. glutamicum* is a Na^+-coupled carrier in contrast to DctA of Gram-negative bacteria and YdbH of *B. subtilis*. Na^+-dependent symport could be characteristic for DctA carriers from the high-GC subgroup of Gram-positive bacteria (Fig. 1).

2.2.3. Genetics and regulation of DctA-related genes

Previously various genes (*dctA*, *dctB* and *cbt*) of *E. coli* were suggested as structural genes for the aerobic C_4-dicarboxylate carrier, including the *cbt* gene for a presumptive C_4-dicarboxylate binding protein [51,52]. But only the function of the *dctA* gene was confirmed [21,40]. A gene cluster (*orfQMP*) with similarity to the *R. capsulatus* binding protein-dependent succinate carrier is found in *E. coli* [21] which, however, is not involved in succinate uptake. The identity and function of the succinate binding protein [51,52] therefore are still not clear.

Synthesis of DctA of *E. coli* is maximal in the stationary growth phase during aerobic growth on succinate or other C_4-dicarboxylates [21]. The C_4-dicarboxylates and citrate cause an about two-fold induction via the DcuSR two-component fumarate regulatory system [13,14]. Glucose repression is about 30-fold and effected by the cAMP–CRP complex which is also responsible for the induction in the stationary phase in an unknown mode [21]. The expression of *dctA* is strongly repressed during anaerobiosis by the ArcBA two-component system.

In contrast, expression of the *dctA* genes of *Sinorhizobium meliloti* and *Rhizobium leguminosarum* is mainly regulated by C_4-dicarboxylates [22,37]. The *dctA* genes of rhizobia are preceded by the *dctBD* genes with divergent orientation to *dctA* [17,22, 24,53]. The *dctBD* genes encode a two-component regulatory system which activates the transcription of *dctA* in the presence of C_4-dicarboxylates. The *dctA* promoter requires the σ-factor σ^{54} for transcriptional activation, DctD and NtrC for signalling

Table 2
Dcu like carriers in facultatively anaerobic bacteria

Bacteria	DcuAB family % identical residues with		DcuC family % identical residues with *E. coli* DcuC		
	DcuA	DcuB	DcuC	DcuD	'DcuE'
E. coli	≡100%	≡100%	≡100%	35	–
P. multocida	–	41[a]	72[c]	37[d]	25[e]
	–	44[b]	–	–	–
H. influenzae	–	42	–	–	23[g]
W. succinogenes	61	71	–	–	–
S. marcescens	91[f]	–	–	–	–
H. pylori	53	–	–	–	–
C. jejuni	53	69	–	–	–
V. cholerae	56	46	61	–	–

The table compares sequences of Dcu proteins derived from databases with the corresponding *E. coli* proteins. In the DcuC family all proteins are compared to DcuC from *E. coli*. –, not present or known.
[a]Orf PM1434.
[b]Orf PM1918.
[c]Orf PM0230.
[d]Orf PM0933.
[e]Orf PM1299.
[f]Only partial sequence.
[g]Orf Y585+Y587.

the presence of C_4-dicarboxylates and N-limitation [54–56].

In *B. subtilis* the expression of the *ydbH* gene encoding the YdbH carrier requires a two-component system consisting of a sensor kinase and a response regulator (*ydbF* and *ydbG* gene products) [25]. Presence of a catabolite-responsive element (CRE) in front of *ydbH* suggests catabolite repression.

2.3. The DcuAB and DcuC carriers

The DcuAB carriers are required for growth by fumarate respiration (Table 2) [1,26,57] and catalyze mainly fumarate:succinate antiport, but uptake and efflux of C_4-dicarboxylates are catalyzed as well [26,58]. DcuC is capable of the same transport activities, but the main function seems to be succinate efflux [27,59]. Due to their similar function the Dcu carriers will be treated together. In *E. coli* the *dcuA* and *dcuB* genes are located adjacent to the *aspA* and *fumB* genes, respectively [26] which encode aspartase A and fumarase B for conversion of aspartate and malate to fumarate in anaerobic growth.

Bacteria often contain more than one *dcu* gene, and the genes are present in various combinations (Table 2). Apart from *E. coli* and *Wolinella succinogenes* [26,29] the different functions of the DcuA and DcuB proteins have not been studied. Carriers of the DcuC family are found in bacteria which produce succinate during glucose fermentation. Most of the bacteria containing *dcuC* homologs are pathogenic. The carriers of the DcuC family can be clustered in two or three subgroups (Fig. 1, Table 2). Only DcuC and DcuD of *E. coli* have been studied [27,28,59].

2.3.1. Properties and topology of the Dcu proteins

From the protein sequence 12 transmembrane helices plus two potential extra helices were predicted for DcuA and DcuB (Table 1) [26]. For DcuA or B consisting of 12 transmembrane helices, the topological model suggests a cytoplasmic orientation of the N- and C-terminal ends, and separation of the protein into two parts by a large central cytoplasmic loop. A model obtained experimentally by *dcuA–blaM* fusions suggests only 10 transmembrane helices for DcuA [60], with a large central loop of 80 residues in the cytoplasm between helices 5 and 6. The N-terminus and the long C-terminus would be located in the periplasm.

2.3.2. Mechanism and energetics of C_4-dicarboxylate transport by the Dcu carriers

The energetics and kinetics of fumarate/succinate antiport has been characterized in *E. coli* wild-type

grown by fumarate respiration where DcuB is the most important carrier and 2.3 times as active as DcuA [58]. Experiments with mutants containing only DcuA, DcuB, or DcuC, showed similar properties for each of the carriers. The K_m values for the C_4-dicarboxylates ($fumarate_{out}$ or $succinate_{out}$) are about 100 μM for the uptake and exchange reactions.

2.3.3. Fumarate:succinate antiport

Fumarate:succinate antiport is the most important function of the DcuAB carriers of *E. coli*. Antiport activity of each of the Dcu carriers exceeds C_4-dicarboxylate uptake or efflux by factors of 2.6–3 (Table 3) [27,58]. The antiport by DcuB is a 1:1 counter-exchange (Eq. 2). In a similar way each of the C_4-dicarboxylates (fumarate, succinate, malate) or aspartate can be exchanged in homologous or heterologous exchange reactions. In the neutral pH range (pH 6–9) divalent C_4-dicarboxylates ($fumarate^{2-}$, $succinate^{2-}$, $malate^{2-}$) are the substrates, whereas the monovalent H-$fumarate^-$ anion prevailed at pH = 6. The exchange is not sensitive to the dissipation of the H^+ potential Δp or constituents of it (the membrane potential $\Delta\Psi$, or the pH difference ΔpH over the membrane), or to the dissipation of a Na^+ potential. Therefore antiport is an electroneutral exchange of C_4-dicarboxylates (Eq. 2). Electroneutral exchange reactions are also found in mutants containing only DcuB, or DcuA, or DcuC [26,27,59].

$$succinate^{2-}_{in} + fumarate^{2-}_{out} \rightleftharpoons fumarate^{2-}_{in} + succinate^{2-}_{out} \quad (2)$$

2.3.4. C_4-dicarboxylate uptake

Net uptake of C_4-dicarboxylates by the Dcu carriers of *E. coli* is observed when no internal counter-substrate is present [26,57,58]. The uptake results in the accumulation of the substrate by factors of ≥ 60. The C_4-dicarboxylates are taken up as divalent ions (e.g. $fumarate^{2-}$) at pH ≥ 6 and as monovalent ions (H-$fumarate^-$) below pH 6. The uptake is an electrogenic H^+:fumarate symport and requires Δp, $\Delta\Psi$, or ΔpH over the membrane (Eq. 3). In *E. coli* the uptake is not inhibited by the Na^+ ionophore nigericin and does not require addition of Na^+ ions. The Δp-dependent C_4-dicarboxylate uptake is also found in mutants containing only DcuA, or DcuB, or DcuC.

$$3H^+_{out} + fumarate^{2-}_{out} \rightarrow 3H^+_{in} + fumarate^{2-}_{out} \quad (3)$$

2.3.5. Succinate efflux

E. coli produces up to 0.2 mol succinate in glucose fermentation which has to be excreted. Efflux is the

Table 3
Contribution of the DctA and Dcu carriers to the transport of C_4-dicarboxylates in *E. coli* during aerobic and anaerobic growth

Transport mode (growth condition)	Transport (U/g dw)		Contribution of single carriers (U/g dw)				Diffusion (U/g PL)
	Wild-type	Theoret. activity	DctA	DcuA	DcuB	DcuC	
Succinate uptake, aerobic (Suc+O_2)	35.0	122[a]	35.0	3.2	≤0.2[d]	≤0.1[d]	0.02
Fum:Suc exchange (Glyc+Fum)	21.8	300[b]	≤0.5	17.1	39.2	5.4	ND
Succinate efflux (glucose)	26.0	143[c]	ND	ND	ND	ND	0.02
Succinate uptake (glucose)	13.9	ND	≤0.2	8.5[d]	3.9[d]	2.7[d]	0.02

The transport activities were measured in the wild-type and in strains containing only the carrier indicated (compare [26,27,35,59]). Where indicated were the activities derived from transport activities and the expression (reporter gene fusions) under the respective conditions. Diffusion refers to carrier-independent transport of C_4-dicarboxylates across lipid membranes (PL) [35]. The theoretical transport activities (a) required for the corresponding growth reaction were calculated from the growth rates (μ) and molar growth yields (Y) under the respective conditions according to $a = \mu/Y$. Activities are given in μmol/min/g dry weight, U/g dw. ND, not determined.

[a]Calculated from $\mu = 0.02\ min^{-1}$, $Y = 45$ g dry weight (mol succinate)$^{-1}$ (Janausch and Unden, unpublished).

[b]Calculated from $\mu = 0.002$ and $0.0026\ min^{-1}$, and $Y = 7$ and 8.4 g dry weight (mol fumarate)$^{-1}$ for growth on H_2+fumarate, or glycerol+fumarate, respectively ([94], Janausch and Unden, unpublished).

[c]Calculated from $\mu = 0.015\ min^{-1}$, $Y = 21$ g dry weight (mol glucose)$^{-1}$ and the formation of 0.2 mol succinate per mol glucose ([35], Janausch and Unden, unpublished).

[d]Calculated from transport rates and the expression of the genes under the respective conditions (compare [13,14,26,27,35,59,64]).

least characterized mode of the anaerobic C_4-dicarboxylate transport due to the lack of appropriate mutants and efflux assays. The efflux of succinate from deenergized membrane vesicles generates a $\Delta\Psi$ of −70 mV (outside positive), suggesting that succinate efflux occurs in symport with H^+ and represents the reversal of the uptake [58]. By this, succinate efflux generates a Δp similar to the efflux of lactate or other fermentation products from bacteria [61]. In *Selenomonas* Na^+-coupled efflux of succinate has been described [6].

In *E. coli* any of the Dcu carriers can contribute to the efflux by a reversal of the uptake reaction [58,59]. Deletion of DcuC stimulated the uptake and exchange activities by DcuA and DcuB, presumably due to decreased efflux. Therefore DcuC seems to be important for succinate efflux. Accordingly, DcuC is produced during glucose fermentation, when DcuB synthesis is repressed. However, deletion of DcuC (in addition to DcuA, DcuB, DcuD, CitT, DctA) in *E. coli* does not inactivate succinate efflux [28,35]. Therefore additional carriers have to participate in succinate efflux.

2.3.6. Model for the function of the Dcu carriers in exchange, uptake and efflux

One striking property of the Dcu carriers is their ability to catalyze exchange, uptake and efflux of C_4-dicarboxylates. The switch in the transport mode can be interpreted in analogy to that of the lactose/H^+ symporter of *E. coli* which is able to perform electrogenic uptake or efflux, and an electroneutral exchange [62]. According to this model (Fig. 2), fumarate^{2-} (or C_4-dicarboxylate^{2-}) uptake by the Dcu carriers of *E. coli*, one H^+ is bound to the carrier before binding of the substrate. Since fumarate uptake is electrogenic, fumarate^{2-} would be transported with two further H^+ across the membrane. In the second part of the cycle, the carrier would return to the start conformation after release of fumarate^{2-} and $2H^+$ in the cytoplasm. In the efflux reaction the sequence of reactions would be reversed, whereas in the exchange reaction one half of the cycle would be used in both directions (Eq. 4). The model explains the different transport modes of the Dcu carriers of *E. coli*, and it is supported by the effects of ΔpH and $\Delta\Psi$ on the uptake and exchange reactions, although ΔpH and $\Delta\Psi$ changes did not relate quantitatively to predicted changes in transport rates [58].

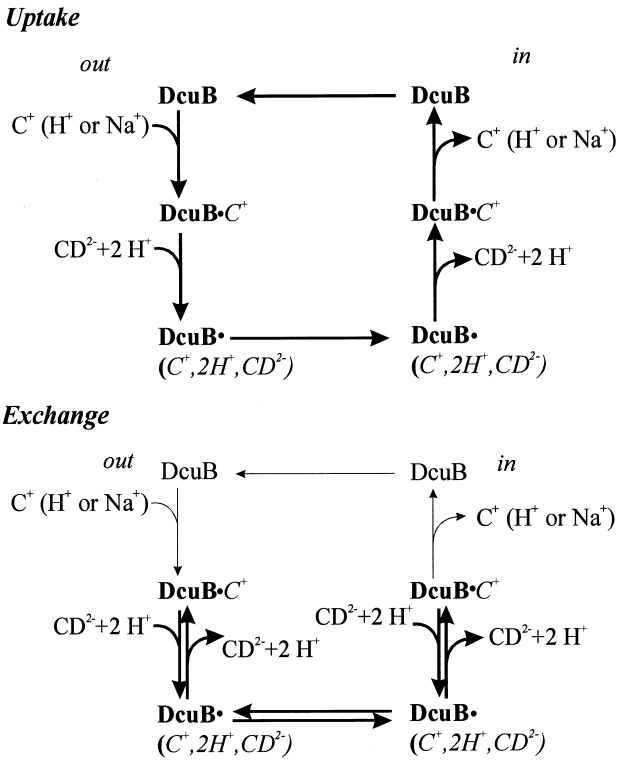

Fig. 2. Model for C_4-dicarboxylate uptake and exchange catalyzed by the DcuB and DcuA carriers of *E. coli*. The model is shown in analogy to the electrogenic uptake and the electroneutral exchange catalyzed by the lactose: H^+ symporter of *E. coli* [62]. *Uptake*: In the electrogenic uptake (H^+:succinate^{2-} symport) sequential binding of a H^+ or Na^+ (=C^+) and of a C_4-dicarboxylate^{2-} (CD^{2-})+$2H^+$ occurs at the carrier to sites accessible from the outside. After orientation of the sites to the cytoplasm, succinate and the H^+ and/or Na^+ are released. For efflux the same sequence of reactions is used in the reverse direction. *Exchange*: For exchange, only the lower part of the reaction sequence (bold) would be used in the forward and backward direction, resulting in an electroneutral exchange. The figure shows a homologous succinate:succinate exchange.

$3H^+_{out}$+fumarate$^{2-}_{out}$	→	$3H^+_{in}$+fumarate$^{2-}_{in}$	(uptake)	(4a)
3 H^+_{in}+succinate$^{2-}_{in}$	→	3 H^+_{out}+succinate$^{2-}_{out}$	(efflux)	(4b)
fumarate$^{2-}_{out}$+succinate$^{2-}_{in}$	→	fumarate$^{2-}_{in}$+succinate$^{2-}_{out}$	(exchange)	(4)

In *W. succinogenes* fumarate:succinate exchange by the Dcu carriers depends on the presence of Na^+ ions [29]. It can be assumed for *W. succinogenes* that (part of) the H^+ of the reaction cycle is replaced by Na^+ ions (Fig. 2).

2.3.7. Regulation of the activity of the Dcu carriers

The activity of the Dcu carriers is subject to transcriptional and post-translational regulation. The post-translational inactivation of the Dcu carriers is caused by electron acceptors like O_2 and can be reversed by reducing agents [57]. A similar inhibition by O_2 has been described for the nitrate carrier of *E. coli* [63].

The expression of the *dcuB* and *dcuC* genes is highly regulated whereas that of *dcuA* is constitutive [59,64]. Expression of *dcuB* is activated under anaerobic conditions by the O_2-dependent regulator FNR, and by fumarate and the fumarate two-component regulator DcuSR. On the other hand, the *dcuB* gene is repressed by nitrate and the nitrate two-component regulatory system NarXL. In addition *dcuB* is subject to CRP-mediated catabolite repression. Overall an 150-fold induction is achieved during growth by fumarate respiration compared to aerobic growth.

Expression of *dcuC*, too, requires activation by FNR under anaerobic conditions. In addition, fumarate stimulates the expression by a factor 2 which, however, does not require the DcuSR system. In contrast to *dcuB* is the expression of *dcuC* only slightly repressed by nitrate and glucose. Thus *dcuC* is expressed under most anaerobic conditions, even in the presence of glucose and nitrate. From *dcuD* of *E. coli* an intact protein can be produced, however, no conditions causing expression of the gene were found, suggesting that *dcuD* codes for a silent gene.

The *dcuA* gene is expressed constitutively, but under aerobiosis the expression decreases to 40% of the aerobic [64]. No specific role could be identified for DcuA so far, and DcuA seems to be a general backup carrier.

2.4. The TRAP carriers

The TRAP carriers are secondary carriers using the electrochemical H^+ gradient as the driving force. In contrast to conventional secondary carriers they require a periplasmic solute receptor binding protein for transport [30–32]. The DctPQM carrier from *R. capsulatus* is used for the uptake of C_4-dicarboxylates as carbon and energy sources, and consists of the DctQ and DctM integral membrane proteins and of the periplasmic solute receptor protein DctP. Homologs of this system are present in other bacteria, but mostly their function has not been studied. When the homologous operon in *E. coli* (*orfQMP*) is inactivated [21], no defects in aerobic growth on succinate are found. It was concluded therefore that the *orfQMP* products play no role in C_4-dicarboxylate transport under these conditions. In *W. succinogenes* *dctPQM* homologous genes have been identified in addition to the DcuA and DcuB carriers. Inactivation of the *W. succinogenes dctPQM* genes had no effect on the growth by fumarate respiration [29]. On the other hand, presence of the DctPQM carrier was sufficient to support growth with succinate as the carbon source during nitrate respiration.

2.5. The CitT carrier

In citrate fermentation *E. coli* converts citrate in the presence of an additional electron donor to succinate and acetate [7,8]. Citrate uptake and succinate efflux are effected by CitT [33]. The bacteria containing CitT catalyze a heterologous exchange of citrate against succinate, fumarate or tartrate, or a homologous exchange of citrate. By this property CitT differs from the other carriers discussed here (DctA, DcuAB, DcuC, DctPQM) which are specific for C_4-dicarboxylates. Growth by anaerobic citrate fermentation is lost in the *citT* mutant, whereas a *dcuA dcuB dcuC* mutant has no effect on growth by citrate fermentation. Aerobic expression of *citT* enables aerobic growth of *E. coli* on citrate which is not found in wild-type *E. coli* due to the lack of a suitable transport system. From these results it is concluded that CitT catalyzes coupled citrate:succinate antiport.

CitT contains 12 predicted transmembrane helices. It represents a new class of bacterial carriers for the transport of di- and tricarboxylates which are related by sequence to the 2-oxoglutarate:malate translocators from spinach chloroplasts. One further bacterial C_4-dicarboxylate carrier of this family is the putative tartrate/succinate antiporter of *E. coli* (*ttdT* gene

product) which is located adjacent to the tartrate dehydratase genes *ttdAB* [33,65].

2.6. Contribution of diffusion to C_4-dicarboxylate transport?

Succinate is able to diffuse across phospholipid membranes if present as a (cyclic) monoanion at acidic pH (pH≤6) [66,67]. If a large concentration gradient of succinate is available over the membrane, a $\Delta\Psi$ can be generated by diffusion of the monoanion along the concentration gradient. However, the diffusion rates of succinate and other C_4-dicarboxylates across membranes are very low compared to the rates of carrier-mediated transport [35] (Table 3). Thus carrier-independent diffusion of succinate at pH 6 is orders of magnitude to slow for the rates required for growth. Accordingly, mutants lacking all carriers for C_4-dicarboxylates are no longer able to grow at neutral pH on succinate significantly.

2.7. Use of alternative C_4-dicarboxylate carriers in E. coli

The presence of at least six different C_4-dicarboxylate carriers (DctA, DcuA, DcuB, DcuC, CitT, and one more efflux carrier) in *E. coli* [21,26–28,33] and of four different C_4-dicarboxylate carriers (DcuA, DcuB, DctPQM, one unidentified uptake carrier) in *W. succinogenes* [29] raises the question for their role in different metabolic pathways. In *E. coli* it was possible to identify the function of most of the carriers by genetic and biochemical studies (Table 3, Fig. 3). Generally the activities of the carriers measured in the bacteria are lower than expected from the metabolic rates and amount to 13–29% of the calculated activities (Table 3). The discrepancy to the required activities might be due to problems with the measurement of initial transport rates of secondary carriers.

The significance of DctA for the uptake of C_4-dicarboxylates during aerobic growth is obvious [21]. *dctA* is expressed maximally in aerobic growth. DctA is the most active carrier under these conditions (Table 3) and a *dctA* mutant shows only low residual growth on succinate [21,35]. DcuA is produced under aerobic conditions, but is of low activity compared to DctA and O_2-sensitive [57]. A low residual succinate uptake in the *dctA* mutant has to be effected by other carriers such as a monocarboxylate carrier [35], KgtP

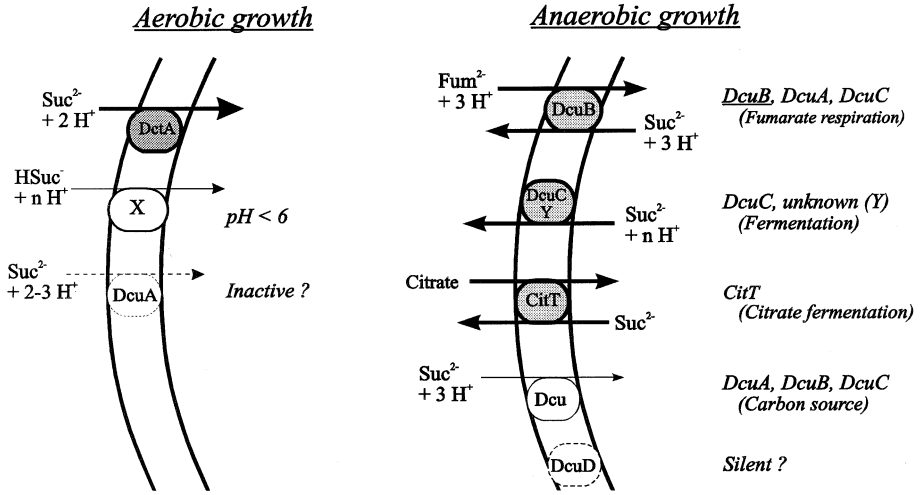

Fig. 3. Carriers and transport mode for the exchange, uptake and efflux of C_4-dicarboxylates in *E. coli* under aerobic and anaerobic growth conditions. The major or catabolic carriers are shaded. *Aerobic growth*: DctA is the major carrier for uptake. In *dctA* mutants an unknown (monocarboxylate?) carrier takes over the function at pH≤6. DcuA is present in low amounts and inactive. *Anaerobic growth*: During growth by *fumarate respiration* DcuB is the major carrier for electroneutral fumarate:succinate antiport. DcuA can be supported by DcuA and DcuC. During *glucose fermentation* succinate efflux is effected by DcuC together with an unknown efflux carrier Y. The carriers may be supported by DcuA. In *citrate fermentation* CitT catalyzes citrate:succinate antiport. DcuA might be used to support the switch between aerobic and anaerobic C_4-dicarboxylate transport as a general support of the other carriers. The *dcuD* gene is silent. See text for details.

[34], the putative SucT carrier [21], or others. The supposed monocarboxylate carrier is able to replace DctA efficiently during aerobic growth at acidic pH [35].

In fumarate respiration, fumarate:succinate exchange is only catalyzed by the Dcu carriers, which are essential for growth by fumarate respiration [26,27]. DcuB or DcuA, but not DcuC, supports full growth by fumarate respiration [26], and DcuB shows the highest antiport activities. Due to the high activity and the specific induction, DcuB is the major fumarate:succinate antiporter for fumarate respiration.

During anaerobic growth by glucose fermentation which requires succinate efflux, only the Dcu carriers are active [27,59]. The expression pattern of *dcuC* and transport activities of *dcuC* mutants indicate that DcuC is required for the efflux activity together with another unknown carrier. The constitutive carrier DcuA presumably contributes to succinate efflux as well. Since an additional carrier masks the activity of the Dcu carriers the contribution of DcuC, DcuA, and DcuB to succinate efflux could not be measured directly.

In summary, specific functions could be identified for DctA, DcuB, and possibly DcuC in uptake of C_4-dicarboxylates during aerobic growth (DctA), C_4-dicarboxylate:succinate antiport during fumarate respiration (DcuB), and succinate efflux during fermentation (DcuC). DcuA on the other hand is synthesized constitutively and might represent a general backup system or a system enabling rapid switch between aerobic and anaerobic growth, or other conditions requiring different types of C_4-dicarboxylate carriers.

3. Sensors for C_4-dicarboxylates

Response of bacteria to C_4-dicarboxylates in the medium depends on typical two-component sensor regulator pairs consisting of a membraneous sensor kinase and a cytoplasmic response regulator [68,69]. In the presence of a signal, kinases of this type autophosphorylate a conserved histidine residue of the sensor. The phosphate group is then transferred to a conserved aspartate residue in the receiver domain of the response regulator.

In chemotaxis of bacteria and archaea cytoplasmic fumarate is also required for signal transmission from the chemotaxis sensor proteins to the flagella motors [70–72]. This role of fumarate in signal transduction is different from the function as an external signal molecule which will be described here.

3.1. Families of C_4-dicarboxylate sensors

The bacterial sensors DcuS, DctB, DctS and Tar for C_4-dicarboxylates and for aspartate can be grouped in four families (Fig. 4). Three of the sensor families contain typical two-component sensors which constitute together with a response regulator a sensor–regulator pair or two-component system: DcuS is a member of the CitA family [13,14,73], DctB of the NtrB family [16,17,74], and DctS of the FixL family [15,75] (Fig. 4). The C_4-dicarboxylate sensors DcuS, DctB, and DctS have similar functions and topologies, but their supposed periplasmic C_4-dicarboxylate binding domains are not related. The fourth sensor, Tar [69,76], is specific for aspartate and represents a methyl-accepting chemotaxis protein which controls chemotaxis in response to environmental signals.

The CitA family of two-component sensors contains various confirmed and putative citrate and C_4-dicarboxylate sensors (Fig. 4). CitA, the first identified member of this family [73], is a citrate sensor in *Klebsiella* and *E. coli* and controls the expression of genes of citrate fermentation. The putative CitA protein from *S. coelicolor* and the CitS citrate sensor from *B. subtilis* [77] (not shown) are also members of the family. The other members of the family are C_4-dicarboxylate sensors. DcuS from *E. coli* shows close similarity (27% identical amino acid residues) to CitA from *Klebsiella*. *B. subtilis* contributes two further sensors to the CitA family, YdbF and YufL [25]. YdbF from the YdbFG sensor–regulator pair functions as a C_4-dicarboxylate sensor [25] which requires the periplasmic succinate binding protein YdbE for succinate sensing. Involvement of periplasmic solute binding proteins is also known for methyl-accepting chemotaxis proteins [69]. The putative YufL sensor kinase from the sensor–regulator pair YufLM (*yufLM* gene products) shows an even higher similarity to DcuS than YdbF, and could represent a further C_4-dicarboxylate sensor.

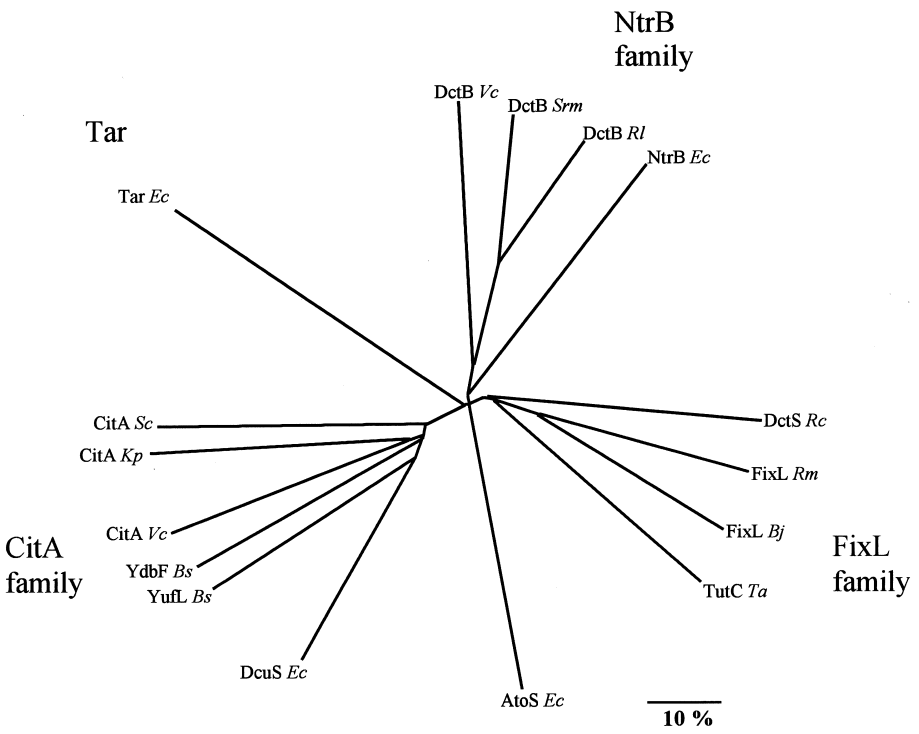

Fig. 4. Relation of the DcuS (CitA family), DctB (NtrB family), and DctS (FixL family) membrane sensors for C_4-dicarboxylates, the Tar sensor for aspartate, and of the AtoS sensor by protein sequence differences. The CitA, NtrB, FixL families and AtoS represent two-component sensors, Tar is a methyl-accepting chemotaxis sensor. The distances give the differences in identical amino acid residues of the sensors or the corresponding (putative) gene products. For the comparison the Clustal W program was used (opening, end, extending and separation gap penalties: 10, 10, 0.05, and 0.05, respectively). Abbreviations of strains: *Bj*, *Bradyrhizobium japonicum*; *Bs*, *Bacillus subtilis*; *Ec*, *Escherichia coli*; *Kp*, *Klebsiella pneumoniae*; *Rc*, *Rhodobacter capsulatus*; *Rl*, *Rhizobium leguminosarum*; *Sc*, *Streptomyces coelicolor*; *Srm*, *Sinorhizobium meliloti*; *Ta*, *Thauera aromatica*; *Vc*, *Vibrio cholerae*.

In the CitA, DcuS, YdbF and YufL proteins the sequence similarities are extended over the complete length of the proteins including the periplasmic sensing domain. This applies also to the periplasmic domain of YdbF which shares 32% identical residues to the domain of DcuS. Therefore the topology identified for CitA and DcuS [14,73] with two transmembrane helices enclosing a long periplasmic domain, and a C-terminal cytoplasmic domain can be expected for all proteins of this class. All of the two-component sensors shown in Fig. 4 interact with corresponding response regulators. The response regulators of DcuS and CitA, DcuR and CitB, are closely related and show 29% sequence identity.

The DctB C_4-dicarboxylate sensors are found in rhizobial strains and show sequence similarity to the NtrB two-component sensor kinases of nitrogen fixation [16,74]. DctB is a membraneous sensor with two supposed transmembrane helices, a periplasmic sensory and a cytoplasmic transmitter domain [17,78–80]. The sequence similarity to the cytoplasmic sensor kinase NtrB applies mainly to the transmitter domains. The response regulator DctD of the DctBD pair shows also clear similarity to the response regulator NtrC of the NtrBC pair, and extends over all functional domains of NtrC. NtrC consists of an N-terminal receiver domain, a C-terminal domain for binding to upstream activator DNA sequences, and a central ATPase domain for transcriptional activation of σ^{54}-dependent promoters which is characteristic of this class of response regulators [54,55,81–83]. The response regulators DcuR and DctR of the other C_4-dicarboxylate regulators lack the central ATPase domain of NtrC and DctD and control the expression of standard (σ^{70})-dependent promoters.

The third type of C_4-dicarboxylate sensors is represented by the DctSR sensor–regulator pair of the purple photosynthetic bacterium *R. capsulatus* [15]. DctSR is required for aerobic growth of the bacteria

on C_4-dicarboxylates. The sensor DctS shows sequence similarity to the oxygen sensor FixL of nitrogen fixation in rhizobia, but no significant similarity to the sensor kinases DcuS and DctB. A corresponding similarity is also seen for the response regulator DctR to FixJ, the response regulator of the FixLJ pair (42% identical residues). The TutC histidine kinase of *Thauera aromatica* which controls anaerobic toluene metabolism is also distantly related to DctS and the FixL proteins [84].

The aspartate sensor Tar controls the chemotactic response of *E. coli* to aspartate [69,76,85]. Tar is a membraneous sensor with a periplasmic sensory domain in a four-helix bundle structure with an aspartate binding site [76,85]. Tar shows no overall sequence similarity to the two-component fumarate sensors DcuS, DctB, or DctS over the entire sequence. Even in the periplasmic signal perceiving domain no significant sequence similarity to the periplasmic domains of DcuS, DctS, and DctB is seen despite the close similarity of the signal molecules.

AtoS of *E. coli* (Fig. 4) is the sensor kinase of the putative sensor–regulator pair encoded by the *atoSC* genes and shows low sequence similarity to DctS, DcuS, and DctB proteins. The *atoSC* genes are located adjacent to the *atoD*, *atoA*, *atoE*, *atoB* genes encoding enzymes related to short-chain fatty acid metabolism. This location and the (low) similarity to a C_4-dicarboxylate sensor suggest that AtoS could be involved in short-chain fatty acid sensing. The *orf28171* gene product from the archaeon *A. fulgidus* shows low similarity to the sensors of the CitA/DcuS and Tar. It is feasible that this protein represents a di- or tricarboxylate sensor histidine protein kinase.

3.2. The rhizobial DctBD two-component system

In rhizobia C_4-dicarboxylates are the major carbon source during symbiotic growth. Growth on the C_4-dicarboxylates requires the DctBD two-component system which was the first identified C_4-dicarboxylate-responsive system [16,86–88].

3.2.1. Genetics and transcriptional activation by DctBD

The C_4-dicarboxylate-dependent regulation by the DctBD two-component system was studied in *S. meliloti* and *R. leguminosarum*. The DctBD sensor–regulator pair controls the expression of the *dctA* gene encoding the succinate uptake carrier DctA. The *dctB*, *dctD* genes are located upstream of *dctA* in divergent orientation.

The DctB–DctD sensor–regulator pair has been characterized mainly by genetic methods. DctB and DctD are similar in function to the NtrB–NtrC nitrogen regulatory two-component system and activate the expression of the σ^{54}-dependent promoter of *dctA* [16]. The σ^{54} binding site (or promoter) of the *dctA* gene is located about 93 bp upstream of the *dctA* start codon in *S. meliloti* and *R. leguminosarum*. About 75 bp upstream of the putative σ^{54} binding site upstream activating sequences (UAS) containing inverted repeats are found [56,89]. The functional domains of DctD have been demonstrated by deletion and mutagenesis analysis [54,55,81–83]. Phosphorylated DctD binds cooperatively to a tandem upstream activator site by a C-terminal helix-turn-helix DNA binding motif. DctD interacts with σ^{54} and the β subunit of RNA polymerase, like NtrC and other σ^{54}-dependent transcriptional regulators. For transcriptional activation ATP is hydrolyzed by the central ATPase domain of DctD, and used to promote open complex formation of the transcriptional complex.

3.2.2. Signal perception and transmission by the sensor DctB

DctB is predicted to have a large periplasmic domain of about 280 amino acid residues which is located between the two transmembrane helices of the protein (Fig. 5) [17,78–80]. The C-terminal part of the protein of >200 amino acid residues shows similarity to sensory histidine protein kinases. The transmitter domain includes a kinase domain and is capable of autophosphorylation, presumably at a conserved His415 residue. It is suggested that after binding of the signal (succinate, fumarate, malate) to the periplasmic sensory domain, the signal is transduced across the membrane, stimulating phosphorylation of the transmitter domain and phosphoryl transfer to DctD. The modular structure of DctB was analyzed by genetic truncation of the protein. A C-terminal fragment comprising the transmitter domain is capable of autophosphorylation and phosphoryl transfer. Removal of 58 amino acid residues from the C-terminus (including segments F and G2

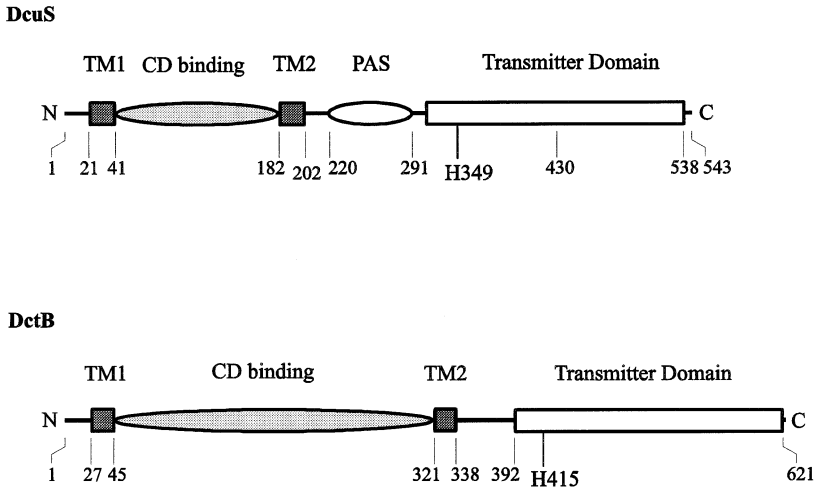

Fig. 5. Predicted domain structure of the DcuS and DctB C_4-dicarboxylate sensors of *E. coli* and *S. meliloti* [14,17,73,78–80] (and SwissProt database http://www.expasy.ch, P39272 and P13633). The position of the domains is taken from the SwissProt database. Cytoplasmic domains are shown in white, periplasmic in gray, and transmembrane helices (TM) in black. CD, C_4-dicarboxylate.

of the transmitter domain) results in a protein incompetent in autophosphorylation.

The presence of the large periplasmic domain suggested binding of the C_4-dicarboxylates to DctB in signal perception in the periplasm. However, genetic deletion of the carrier DctA caused constitutive transcription of *dctA*, demonstrating that DctA participates in C_4-dicarboxylate sensing by DctBD [37,88]. It is suggested that DctA contributes directly to signal perception (Fig. 6). In the absence of C_4-dicarboxylates, free DctA is supposed to interact with DctB and to prevent activation (phosphorylation) of DctB. Binding of C_4-dicarboxylates by DctA would interrupt the interaction between DctA and DctB, and release DctB which becomes thus activated. According to this assumption, presence of released (free) DctB would indicate presence of C_4-dicarboxylates, and in a *dctA* mutant DctB would be always in the released and active state.

Despite the significance of DctA for C_4-dicarboxylate sensing, the C_4-dicarboxylates are sensed in addition directly by DctB [80] (Fig. 6). A detailed analysis showed that DctB-dependent expression responds to the C_4-dicarboxylates also in the absence of DctA. However, in the absence of DctA, the sensitivity to the C_4-dicarboxylates was lower, and the DctB-dependent expression had a broader substrate specificity and responded also to osmotic stress.

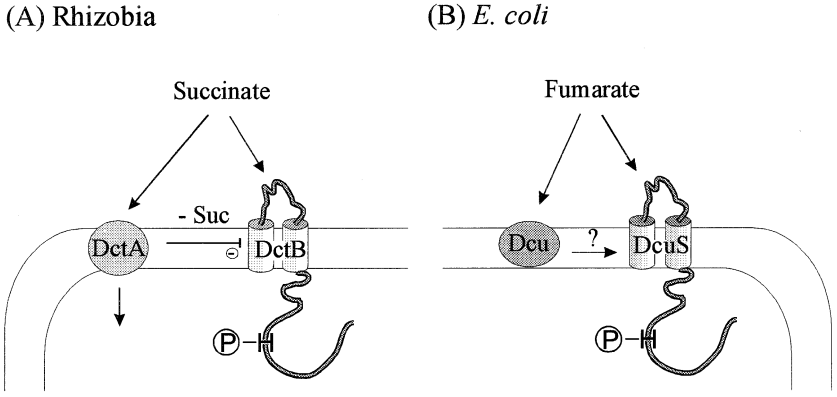

Fig. 6. Scheme for the interaction of extracellular succinate (Suc^-) or fumarate in signal perception (A) by the DctB sensor of *S. meliloti* or *R. leguminosarum* and (B) the DcuS sensor of *E. coli*. In signal perception by DctB the DctA carrier plays an important role in addition to the periplasmic domain of DctB. For signal perception by DcuS, the periplasmic domain is essential for direct interaction with the signalling C_4-dicarboxylates. See text for more details.

Therefore it appears that DctB is a direct sensor for C_4-dicarboxylates, but DctA is required for proper signal detection and signalling specificity.

3.3. The DcuSR two-component system of E. coli

E. coli uses C_4-dicarboxylates (succinate, fumarate, malate, aspartate) as a carbon and energy source under aerobic and aerobic conditions. The enzymes for the corresponding pathways are induced in the presence of C_4-dicarboxylates. Fumarate respiration requires induction of fumarate reductase (*frdABCD* genes), the anaerobic C_4-dicarboxylate carrier DcuB (*dcuB* gene), and anaerobically induced fumarase (*fumB*) and aspartase (*aspA*) genes for the conversion of malate and aspartate to fumarate. In aerobic growth, the C_4-dicarboxylates are oxidized to CO_2 requiring increased induction of the DctA carrier (*dctA* gene), of succinate dehydrogenase (*sdhCDAB*), and of the malic enzyme. In addition, other genes like the *nuoA-N* operon encoding the coupling NADH dehydrogenase, and *dcuC*, also are induced during growth on C_4-dicarboxylates (see [13,14,59, 90]).

3.3.1. Genetics and regulation

The DcuSR two-component sensor–regulator pair controls gene expression in response to C_4-dicarboxylates. The *dcuSR* genes are located upstream of *dcuB* encoding the fumarate:succinate antiporter DcuB [13,14]. By inactivation of the *dcuS* or *dcuR* genes, the *dcuB*, *sdhC*, *frdA* and *dctA* genes showed decreased expression and loss of stimulation by the C_4-dicarboxylates, demonstrating that DcuSR are positive regulators of the target genes. The expression of *dcuB* under anaerobic conditions depended nearly completely on DcuS and the C_4-dicarboxylate, whereas the induction of the *sdhC*, *frdA*, and *dctA* genes or operons much lower. The C_4-dicarboxylate-dependent induction of the *nuoA-N* and the *dcuC* genes was not affected in the *dcuS* or *dcuR* mutants suggesting that additional forms of C_4-dicarboxylate-dependent regulation are present [13].

3.3.2. Properties of the DcuS sensor

The DcuS protein is closely related by sequence similarity to the CitA citrate sensor of *E. coli* and *Klebsiella*. The sensors of the CitA family show all a similar predicted structure consisting of two transmembrane helices in the N-terminal half of the protein (Fig. 5). The domain in between the transmembrane helices is periplasmic which was confirmed by topological studies using *dcuS′–blaM* fusion proteins [14]. The long C-terminal region of DcuS (residues 330–543) is located in the cytoplasm and shows strong sequence similarity to transmitter domains of histidine protein kinases. The domain contains the typical sequence motifs of kinase domains (H, N, G1, F, G2 regions) including His residue 349 which is conserved in all sensors of the CitA family and presumably phosphorylated in the signalling process [13,14].

An extra domain of about 120 amino acid residues is located between transmembrane helix 2 and the C-terminal kinase or transmitter domain [13,14]. The sequence of this domain shows clear similarity to the sensory PAS domains found also in many sensors outside the CitA/DcuS family [14]. PAS domains are known from a large number of eukaryotic and prokaryotic sensor proteins involved in the sensing of light, electron acceptors or electron flow in respiratory chains and of redox status [91]. Many of the PAS domains contain prosthetic groups (FAD, FeS centers, heme groups, and others) for signal sensing. The mode of signal perception by the bacterial PAS proteins is unknown, but it is suggested that direct interaction with enzymes supplying the signal, such as oxidoreductases from the electron transfer chain, are required for signal perception.

3.3.3. Signal perception by DcuS: direct interaction with C_4-dicarboxylates or involvement of additional components?

Regulation by DcuS responds to the C_4-dicarboxylates fumarate, succinate, malate, tartrate and maleate, and the C_4-dicarboxylic amino acid aspartate. Butyrate and other monocarboxylates have no effect. Maleate which efficiently stimulates DcuS-dependent gene regulation, is not taken up and metabolized, or formed endogenously by *E. coli* [13]. Therefore maleate and the other C_4-dicarboxylates have to function directly and without conversion directly at DcuS at the periplasmic aspect of the membrane. Similar conclusions were drawn from experiments with *dcu* mutants which are not able to transport C_4-dicarboxylates during anaerobic growth [14].

The periplasmic domain of DcuS is predestined for signal perception in the periplasm, and there are strong arguments for direct signal perception by this domain. The first is the function of maleate and other C_4-dicarboxylates as the signals from the outside. In addition, maleate apparently even does not interact with or bind to the Dcu carriers since it is no competitive inhibitor of C_4-dicarboxylate transport [26]. Therefore maleate presumably functions by interaction with DcuS (Fig. 6). Secondly, the K_D for C_4-dicarboxylate-dependent regulation by DcuS is very similar to the K_D of C_4-dicarboxylate binding to the isolated periplasmic DcuS domain (Unden and Griesinger, unpublished). Similarly, citrate binds with high affinity (K_D = 5 μM at pH 7) and specificity to the isolated periplasmic domain of CitA from *Klebsiella pneumoniae* without the need for other proteins [92]. Determining the structure of the periplasmic domain of DcuS [93] and of binding of C_4-dicarboxylates to the periplasmic domain should provide direct information on the properties of the ligand binding to the periplasmic domain. Therefore signal perception by DcuS would be different from DctB where the DctA carrier serves an important role (Fig. 6).

There are indications, however, that signal perception by DcuS does not only depend on the periplasmic domain, and the presence of the PAS domain suggests an additional way of signal input into DcuS [14]. The nature of the signal and the enzyme system supplying the signal to PAS are not known, but it has been suggested that the PAS domain of DcuS could respond to electron acceptors like oxygen or nitrate, or to the carbon source. In addition, it has been observed that the expression of *dcuB′-′lacZ* is increased in a *dcuA dcuB dcuC* mutant strain even in the absence of fumarate (Zientz and Unden, unpublished). This could indicate that C_4-dicarboxylate sensing requires in some way the presence of C_4-dicarboxylate carriers. Whether this carrier-dependent regulation requires the function of the PAS domain is not known. Therefore it appears that signal perception by DcuS is a complex process, but it might be an interesting system to study signal perception and transmission in two-component sensors.

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